Biosecurity Plan

MONTAUK HATCHERY

2009

Mill Raceway System

Lake Raceway System

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Hatchery Manager Date

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Hatchery Systems Manager Date

Introduction

The Missouri Department of Conservation's (MDC) Montauk Hatchery is located at the headwaters of the Current River within Montauk State Park in Dent County, Missouri. The hatchery consists of four individual systems, each supplemented with bulk oxygen injection systems (Figures 1-5). Montauk Hatchery's annual production is 350,000 to 400,000 rainbow trout (*Oncorhynchus mykiss*), equaling 300,000 to 350,000 pounds. Montauk produces trout for Montauk State Park, James Foundation's Maramec Spring Park, the Current River, Eleven Point, Roubidoux Creek, Little Piney and Stone Mill Spring White Ribbon Trout Management Areas and St. Louis Winter Urban Fishing Areas.

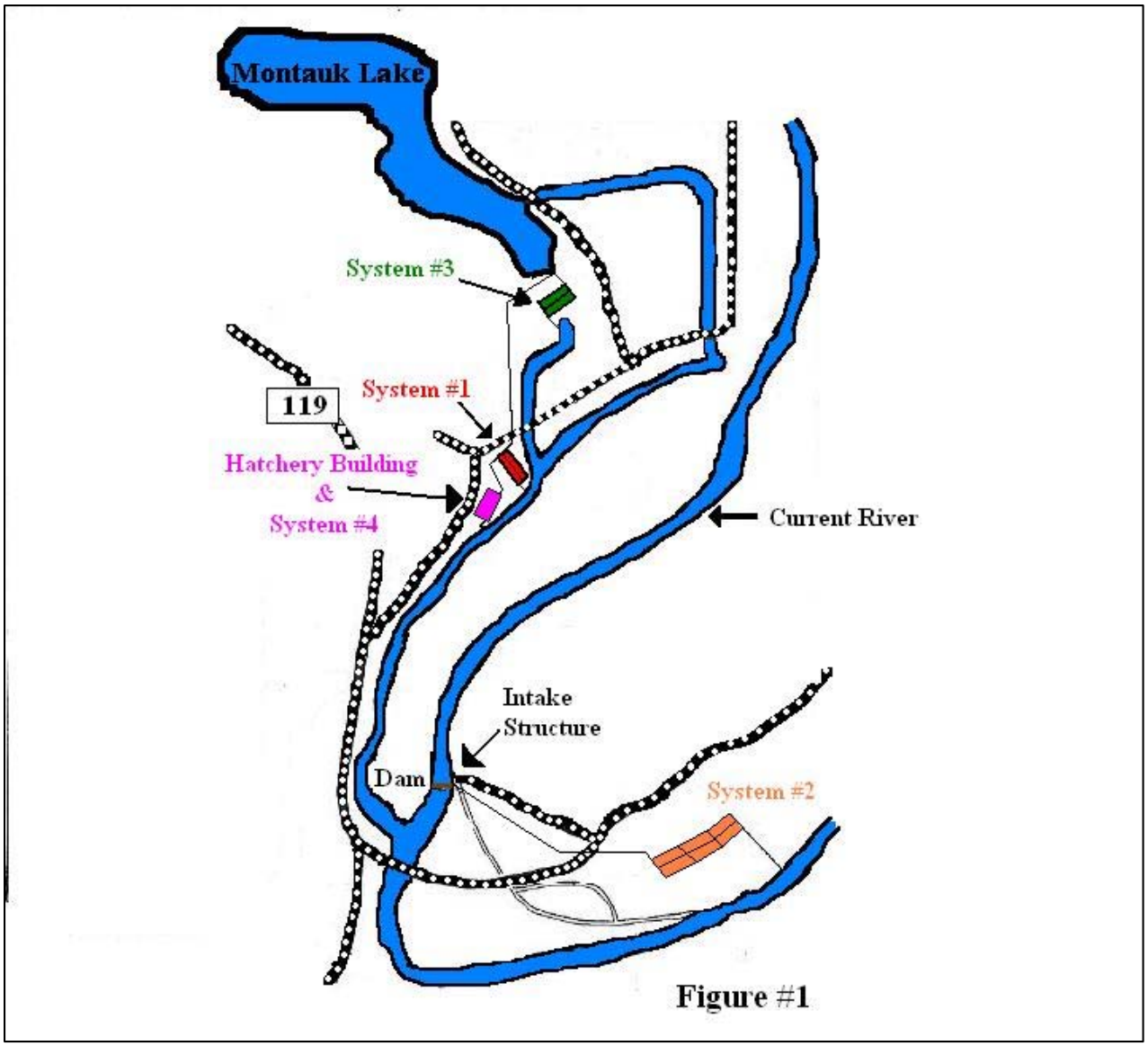

Figure 1. Layout of Montauk Hatchery

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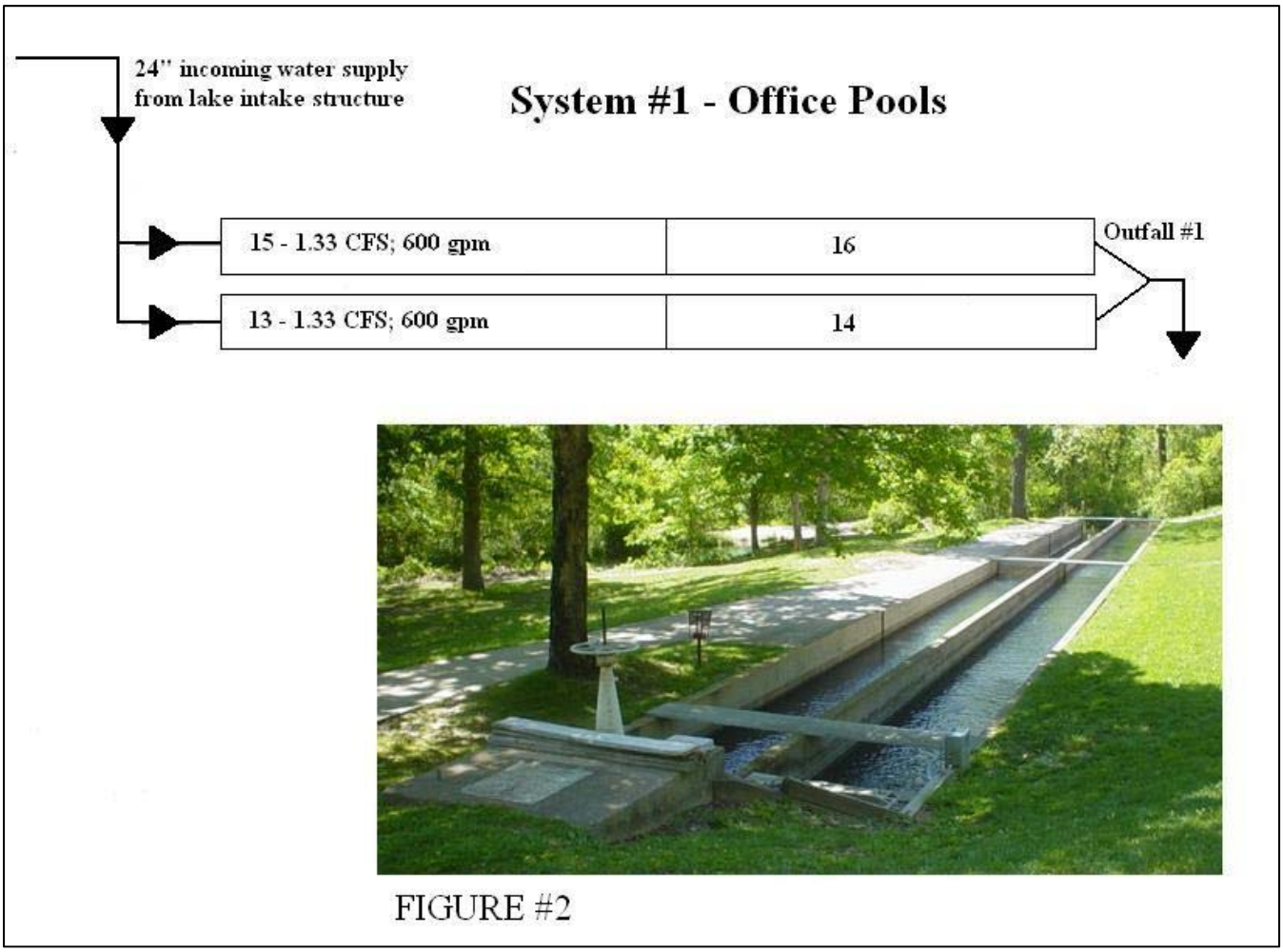

FIGURE #2

Figure 2. System #1 (Office Raceways) consists of two, 150' long by 7' wide raceways with each raceway consisting of two pools (Figures 1 and 2). Bluff Spring (Montauk Lake) is the water supply for this system. System #1 provides 5,102 cubic feet of rearing space and requires 2.66 cubic feet per second (cfs) of water flow. This system is used for fingerling production of rainbow trout (*Oncorhynchus mykiss).*

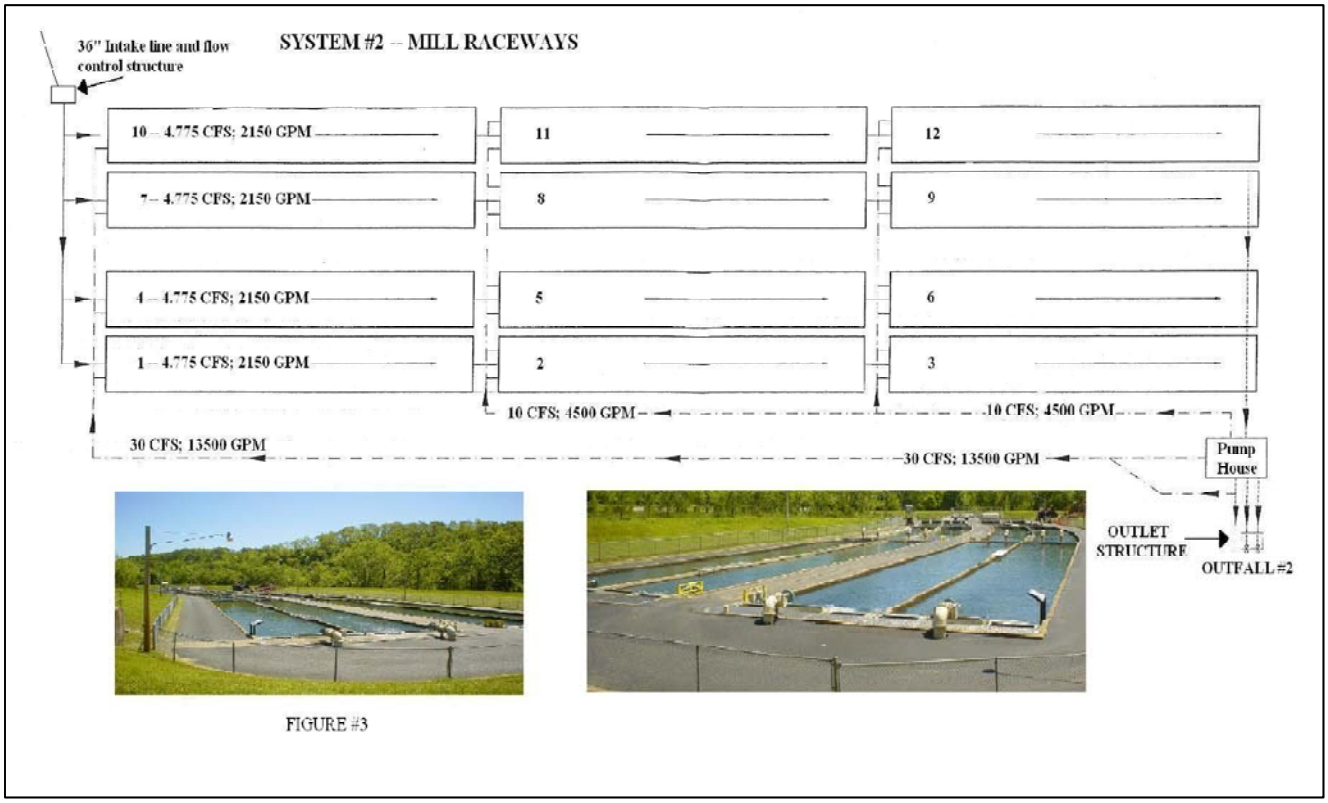

FIGURE #3

Figure 3. System #2 (Mill Raceways) consists of four, 450' long by 20' wide raceways with each raceway consisting of three pools. This system provides 93,000 cubic feet of rearing space and requires 25 to 30 cfs of water flow and is supplied with water from the Current River. This system is used for the bulk of production which consists of rainbow trout grown to a stocking size of 12.5+ inches.

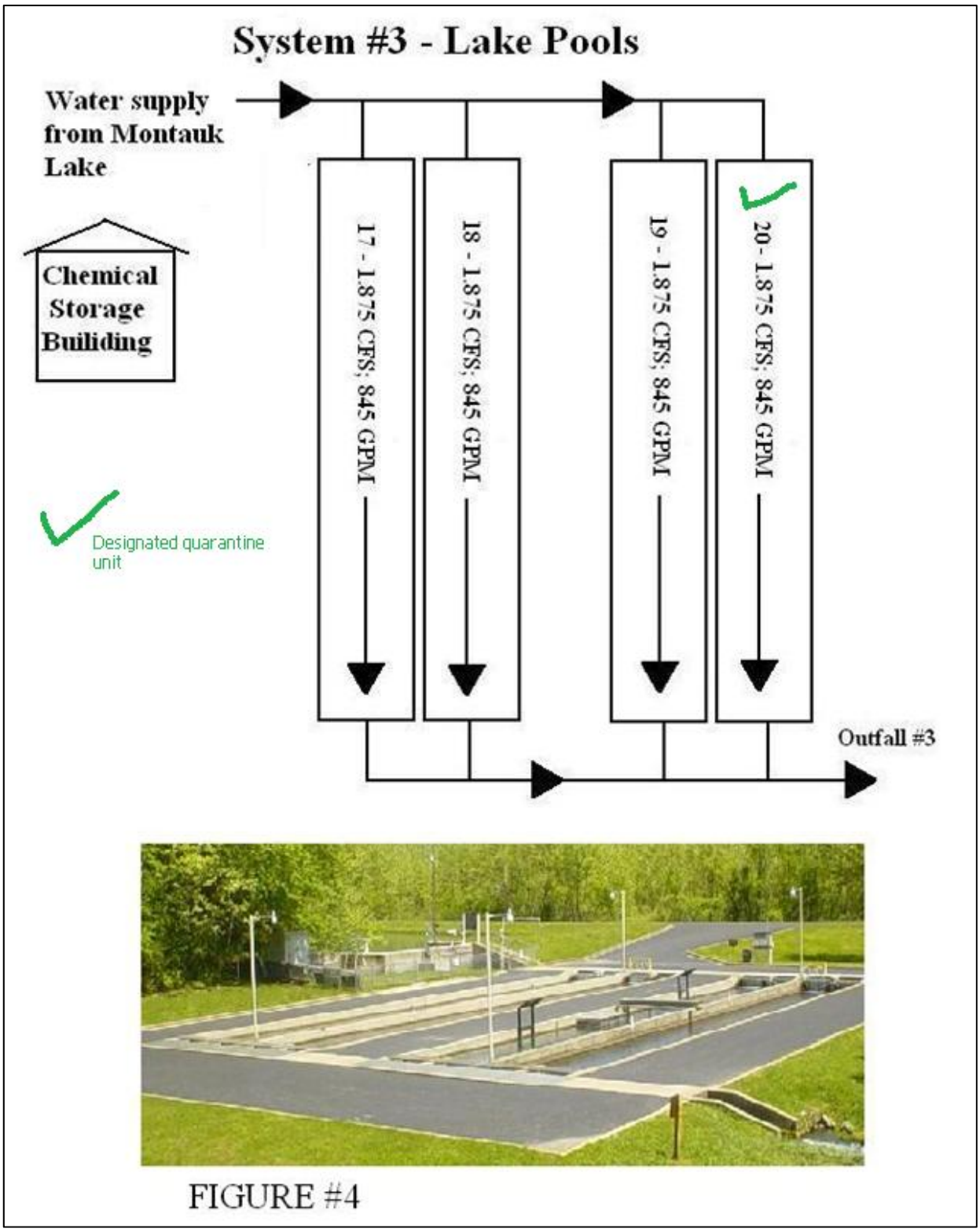

FIGURE #4

Figure 4. System #3 (Lake Raceways) consists of four, 100' long by 12' wide raceways with each raceway consisting of only one pool. This system provides 10,800 cubic feet of rearing space and requires 7.5 cfs of water flow and is fed from the Bluff Spring (Montauk Lake). This system is used for fingerling production of rainbow trout and houses broodstock throughout the year. Raceway 20 is designated as a quarantine unit. All water used in this system is single pass.

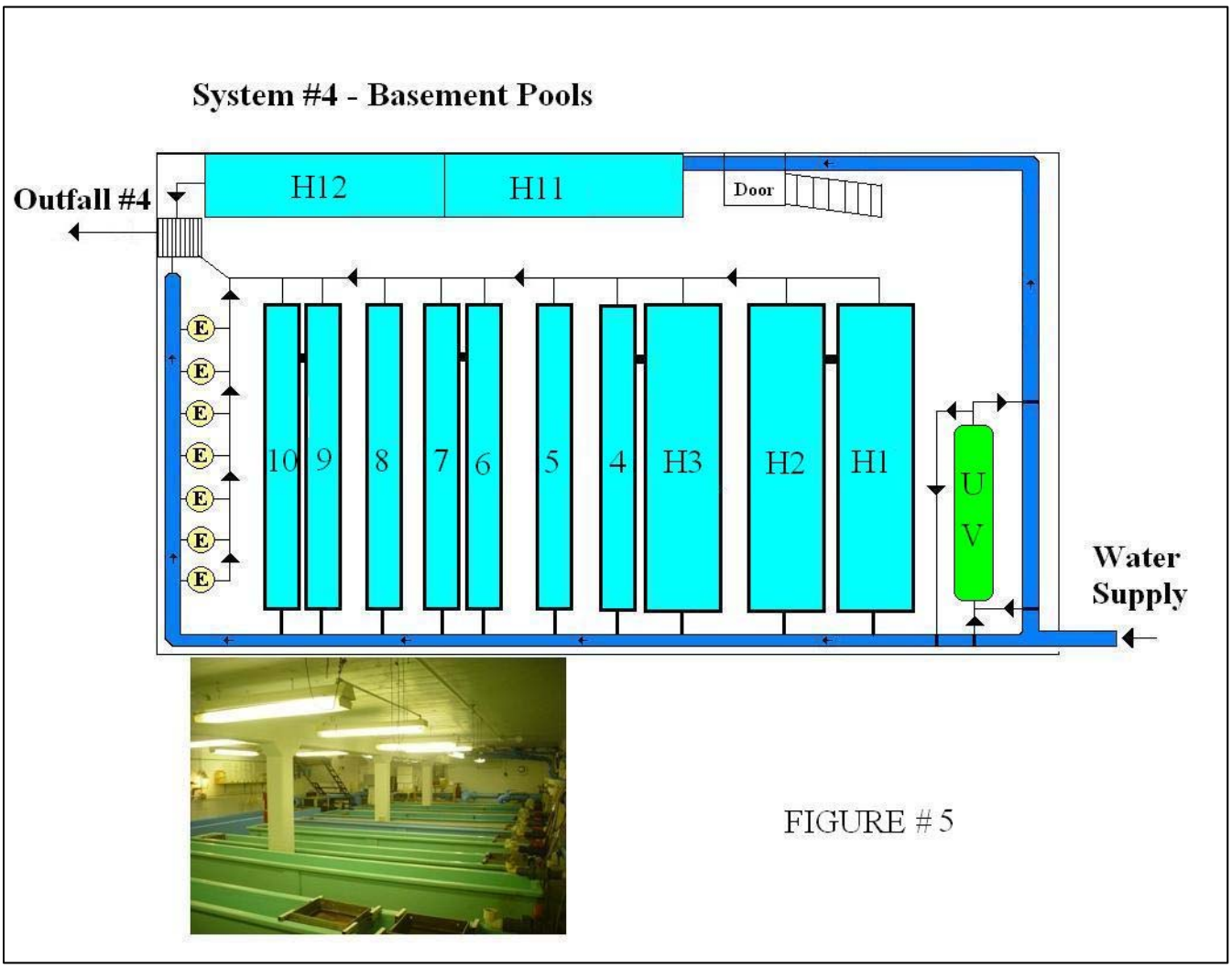

FIGURE # 5

Figure 5. System #4 (Hatchery Building Basement) consists of one, 107 cubic feet concrete tank, three 60 cubic feet fiberglass tanks, seven 11.5 cubic feet fiberglass tanks and fourteen McDonald egg hatching jars . This system is used for eggs, fry and fingerling production of rainbow trout. The water to this system is supplied from the Bluff Spring (Montauk Lake) and is sterilized with a UV unit as it enters the building. This system requires 300 gallons per minute (gpm) flow. When all rearing units are being utilized, the UV unit severely restricts water flow and has to be turned off; hence, it is not able to be used for its designated purpose. A second unit or a larger unit is needed to provide proper treatment of incoming water during periods of high water demand.

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Background and Purpose of Plan

There are two main objectives of the Montauk Biosecurity Plan. The first will be to reduce the chance of importing or exporting pathogens to or from other hatcheries and waters. The second is to improve the ability to reduce disease outbreaks, isolate pathogens and reduce the risk of spreading them throughout the system.

Specific threats to Montauk Hatchery are parasitic copepods, whirling disease, *Costia*, *Trichodina*, *Chilodonella*, *Epistylis*, *Gyrodactylus*, ich, zebra mussels, enteric redmouth, furunculosis, bacterial gill disease, coldwater disease, bacterial kidney disease, columnaris, IPN, IHN and VHS.

Systems #1, #2 and #3 are all exposed to humans and wildlife. Due to the size and location of these systems, there are no feasible means to eliminate this kind of interaction (Figures 1-4). There are opportunities to educate the public on the importance of biosecurity to the Montauk Hatchery system. Most of our focus on these three systems will be to reduce the chance of importing or exporting pathogens to or from other hatcheries or waters, and to improve the ability to reduce disease outbreaks, isolate pathogens and reduce the risk of spreading them throughout the system.

The hatchery basement (System #4, Figure 5) is the area that we have the greatest control of interaction with the public and the ability to eliminate any interaction with wildlife.

Plan Components and Procedures

Following are protocols that will be implemented to improve biosecurity at Montauk Hatchery.

General Equipment Use and Cleaning

Background: A variety of equipment is used at Montauk Hatchery. Equipment and human hands are recognized as modes for pathogen transfer. Viruses, bacteria and parasites are invisible to the naked eye so their transmission via objects goes easily unnoticed. Table 1 provides some medications and disinfectants commonly used in aquaculture. Examples of common equipment items are listed below.

1. Personal protective equipment: e.g., waders, hip boots, rubber boots, raingear, gloves.

2. Work equipment: e.g., dip nets, buckets, brooms, brushes, sponges, towels, feed blowers, aerators, water pumps, weighing scales, mort sticks.

3. Vehicle equipment: e.g., fish trucks, golf carts, pick-up trucks, boats, fish loading pumps, fork lift.

Highest Risks

1. Pathogens: bacterial, viral, parasitic

2. Aquatic nuisance species

General Guidelines

1. The sharing of personal protective and work equipment between fish hatcheries is discouraged. Guest workers at Montauk Hatchery will be provided personal protective equipment to use during their visit, if possible. This equipment shall stay at this site. If there is not enough equipment available for guests and they must bring their own, these items will be thoroughly disinfected before and after use on-site.

2. Equipment and protective clothing will be cleaned or disinfected behind the hatchery building (Figure 6). Any discharge to the stream that could occur from this location would be below the water supply for all four rearing systems.

3. During cleaning the layers of fish slime, mud or organic debris will first be removed through brushing, hosing or power washing, and then they shall be soaked or sprayed with disinfectant.

4. Porous materials, such as wooden handles on dip nets, shall be eliminated and replaced with non-porous materials such as fiberglass or metal. If sponges or cloth towels are used, they shall be clean, changed daily or disinfected after each use.

5. Equipment items will be stored at their location of use. System #1 equipment will be stored in a designated area in the hatchery building. System #2 equipment will be stored in the pump house (Figure 3). System #3 equipment will be stored in the chemical storage building (Figure 4). System #4 equipment will be stored in the basement area of the hatchery building (Figure 5). Equipment will be labeled and used only for that designated rearing unit. Equipment that has to be shared between systems such as fish pumps and waders will be cleaned and disinfected before use.

6. Virkon® Aquatic is the disinfectant of choice because it is the only disinfectant approved for aquaculture use.

7. The potency of reconstituted disinfectants shall be tested at least once every 4 days with test strips. If it has evaporated or the active ingredient level is less than the recommended strength, it shall be refreshed or replaced with new disinfectant.

Vehicle Disinfection

1. If fish transport trucks are sent off-site to deliver or move fish they will be power washed and disinfected either en-route back to Montauk Hatchery or at the designated cleaning site behind the hatchery building (Figure 6). Any discharge to the stream that could occur from this location would be below the water supply of all four rearing systems. They will not be used for any additional loads until they are disinfected. Proper disinfection shall include power washing followed by Virkon® Aquatic 0.5% sprayed inside the holding tank, on nets and through hoses. External surfaces may be further cleaned with 200 ppm chlorine bleach. After disinfection, all equipment will be thoroughly rinsed with clean water.

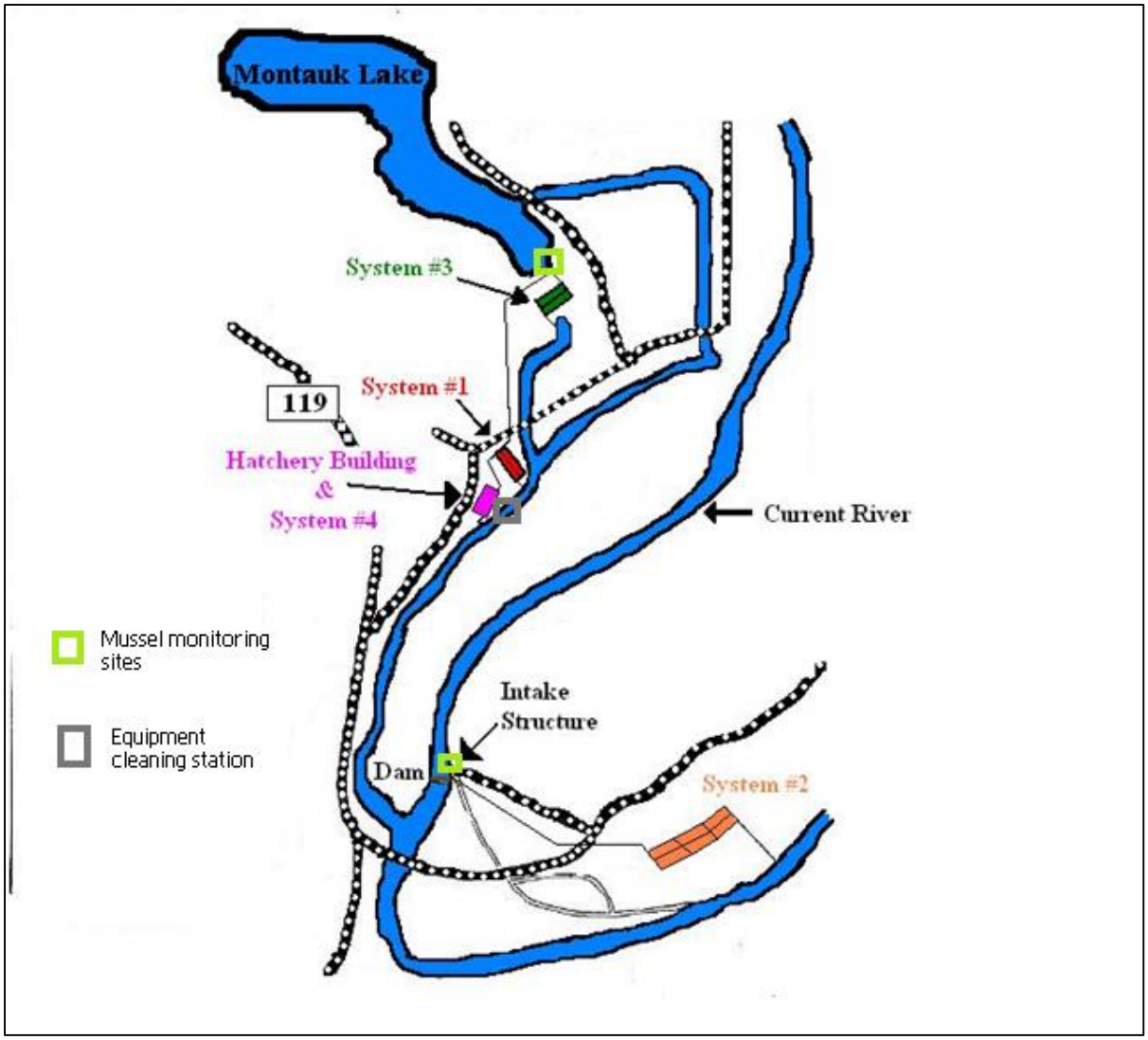


Figure 6. Locations of equipment cleaning station and proposed zebra mussel monitoring sites at Montauk Hatchery.

2. Vehicles used to transport dead fish from raceways to the mort pit area shall be hosed out at the designated equipment cleaning area behind the hatchery building at least once weekly and disinfected (Figure 6). More frequent cleaning will be warranted if mortalities are high or there is spillage from buckets on to the bed of the vehicle.

Table 1. Cost of disinfectants and chemotherapeutics commonly used in aquaculture.

Products	Use/supplier	Contact Time/product	Cost
General Methods			
Hot water-power wash	General cleaning		
Steam clean	General cleaning	5 minutes	
Chemical Treatments			
Formalin 25 ppm	Zebra mussel	KCl-1 hr, then add formalin-	
KCL 750 ppm	prevention	2 hrs = total time of 3 hrs	
Virkon® Aquatic	Western Chemical		10 lb tub= $92 10 lb (4)=$328
0.5%	General use, clothing, inside hauling tanks, equip., vehicles	10-30 min.	
1.0%	Foot bath	10 min.	replenish/check every 4 days
2%	Vaccination equipment	5 min.-then rinse with water	
Argentyne, Ovadine®	Egg disinfection	30 min-water hardening;	1 gallon
50-100 ppm	Argent, Western Chemical	10 min. after water hardening (eyed eggs)	Ovadine® $25.95
Sodium hypochlorite (household bleach)	General cleaning	corrosive to metal, toxic to fish unless detoxified with sodium thiosulfate	
200 ppm	Vehicles	1 hour	
500 ppm	laboratory-counters/floors	10 min-1 hour	
Sodium thiosulfate	detoxify chlorine	grams thiosulfate= 2.85 x grams chlorine used	
Monitoring Supplies			
Chlorine test strips	HACH	0-600 mg/l Cl2 #2890200	q=100 $17.40
	Fisher	0-200 ppm #22-479-808	q=200 $5.15
Iodine test strips	Fisher	0-100 ppm #22-307-863	q=200 $5.15
Virkon®S test strip	Western Chemical-Antec International	see www/wchemical.com	$6.95, or free with each case of Virkon®

Fish Transfers

Background: Fish are stocked into public waters, received from non-MDC hatcheries, captured from the wild, moved between MDC hatcheries and moved within individual hatcheries as they grow and have changing space requirements. Montauk Hatchery annually produces 300,000 to 350,000 pounds of rainbow trout and occasionally will hold up to 4,000 pounds of brown trout (*Salmo trutta)* for stocking the Current River Blue Ribbon Management area and St. Louis Winter Urban Fishing areas. Table 2 summarizes Montauk's typical annual stocking commitments. In the past, Montauk has exported trout and/or eggs to all of MDC's coldwater hatcheries and received trout and/or eggs from all of the hatcheries, except Maramec Spring Hatchery. On rare occasions, eggs are received from a federal hatchery and we may occasionally receive either eggs or fish from private hatcheries. In 2008, for example, 175,000 eggs were received from Erwin NFH, Erwin, TN. Pathogen transfer may occur during all of these activities.

Table 2. Montauk Hatchery annual stocking commitments.

Area	# Trout	# Pounds
Montauk State Park	214,000	186,000
Maramec Spring Hatchery	60,000	52,000
St. Louis Winter Urban	37,000	32,000
Current River White Ribbon Area	8,000	6,900
Stone Mill White Ribbon Area	7,500	4,800
Little Piney Creek White Ribbon Area	2,100	1,800
Roubidoux Creek White Ribbon Area	6,500	5,600
Eleven Point River White Ribbon Area	16,000	13,000
Eleven Point River Blue Ribbon Area	5,500	4,700

Highest Risks

1. Aquatic nuisance species and pathogens (parasitic, bacterial, viral)

    a. Wild fish (unknown health status)

    b. Non-MDC hatcheries

    c. Between MDC hatcheries

Wild Fish Transfers

1. Any wild fish brought on to the hatchery grounds will be placed in isolation and not co-mingled with established hatchery populations unless they pass 6 weeks of quarantine in good health and a sample of fish from this population is found negative for VHS, IPN, IHN, whirling disease, *Aeromonas salmonicida, Yersinia ruckeri* and *Renibacteium salmoninarum* by the Aquatic Animal Health Specialist.

2. Pool #20 in system #3 will serve as the isolation unit, if available (Figure 4). If Pool #20 is not available, the fish will be placed at the tail end of a raceway. This area will have dedicated equipment and supplies used only for this area.

3. Unhealthy wild fish or a representative sample of the population shall be evaluated and treated for parasites and diseases detected during quarantine or they will be culled.

4. Only healthy wild fish will be utilized for broodstock or other purposes.

5. All equipment used on wild fish in quarantine shall not leave the quarantine area and will be thoroughly disinfected with Virkon®Aquatic after use. Any vehicles used to transport wild fish will similarly be cleaned and disinfected after use (see detail above).

5. All eggs obtained from wild fish will be disinfected with 50-100 ppm iodine (Argentyne or Ovadine®) for 15-30 minutes during water hardening. The pH shall be maintained at 7.0-7.5. Appendix 3 provides a detailed procedure for the disinfection of eggs.

6. If *A. salmonicida* is a concern, a second disinfection using 100 ppm for 10 minutes during the eyed stage will be performed.

Transfers from non-MDC hatcheries

1. Before a shipment of Salmonid fish or eggs is received from a non-MDC facility, the shipper shall submit a current fish health inspection record to the Aquatic Animal Health Specialist for review and issuance of an import permit (if necessary).

2. In addition to inspection of this report, the hatchery manager will request the shipper to complete and FAX or e-mail a "Fish Transfer Information Sheet" which shall include mortality records for the previous 15 days (Appendix 1). This sheet will help us identify in writing any other pathogens/nuisance species not specified on the health inspection record which may occur in their watershed or hatchery. This would include things like other viruses, parasites, zebra mussels, New Zealand mud snails, quagga mussels, rusty crayfish or parasitic copepods.

3. Newly imported eyed eggs shall be disinfected with 100 ppm iodine (Argentyne or Ovadine®) for 10 minutes and the shipping container shall be returned to the shipper or sprayed with 500 ppm bleach and placed in trash for disposal.

4. If fish are received, they shall be "quarantined" for at least 4 weeks and observed daily for signs of disease. Fish will be off-loaded in to Pool #20 in system #3 (Figure 4). When possible, this raceway will be an "isolation" unit where the fish can be monitored for and treated for post-transport disease outbreaks before they are co-mingled with other resident fish. If Pool #20 is not available, fish will be off-loaded at the tail of the receiving raceway.

5. All equipment used during the off-loading of these fish shall be immediately disinfected after use (see above).

Transfer of fish between MDC hatcheries

1. Three (3) days prior to the transfer of fish between MDC hatcheries, the shipping facility shall FAX or e-mail to the receiving hatchery manager a “Fish Transfer Information Sheet” which will include a record of the unit’s mortality record for the previous 15 days, comments on the general history of each lot, past chemical therapies and notes on any abnormal behaviors (Appendix 1).

2. Visibly abnormal fish shall be culled prior to shipment.

3. During transport fish shall be treated for Zebra Mussels per MDC policy (Appendix 2):

- 750 ppm KCL for 1 hour, then 25 ppm formalin is added for an additional 2 hours; and

- Total treatment time is 3 hours.

4. If fish are received, they shall be "quarantined" for at least 4 weeks and observed daily for signs of disease. Fish will be off-loaded in to Pool #20 in system #3 (Figure 4). When possible, this raceway will be an "isolation" unit where the fish can be monitored for and treated for post-transport disease outbreaks before they are co-mingled with other resident fish. If Pool #20 is not available, fish will be off-loaded at the tail of the receiving raceway.

5. Post-transportation stress in the fish may be reduced by providing 0.1- 0.5% salt for 1-3 days after arrival.

6. If eyed eggs are received from another MDC hatchery they will be rehydrated for 30 minutes in a bucket of water then disinfected with 100 ppm iodine (Argentyne or Ovadine®) for 10 minutes. The shipping container shall be returned to the shipper or sprayed with 500 ppm bleach and placed in trash for disposal.

7. Rubber boots and raingear worn during delivery of fish shall be sprayed with disinfectant before staff leaves the receiving facility.

8. The transport truck shall be power washed and disinfected either en-route back to the hatchery of origin or at the designated hatchery cleaning site. It shall not be used for any additional loads unless it is disinfected.

Treating Sick Fish

Background: When disease outbreaks occur in a rearing unit the risk of spreading this pathogen to other rearing units increases. Our goal is to isolate this "sick" unit as much as possible.

Highest Risk

1. Spreading pathogen to other rearing units on-site.

General Guidelines

1. The cause of increased mortality shall be identified through necropsy, skin scrape, gill biopsy, clinical signs, bacterial or viral culture. Once the causative agent is identified, appropriate treatment will begin promptly.

2. Units of sick fish will be considered as "quarantine areas" and specific equipment shall be dedicated for their use only. This equipment will stay at that site.

3. All equipment coming in contact with these fish and unit shall be immediately disinfected with 0.5-1% Virkon® Aquatic. It would be helpful to have a bucket of this disinfectant next to the affected raceway.

4. The fish shall not be moved/ transported off-site unless it is determined that this is necessary for their therapy. If moved to another hatchery, appropriate treatment and mortality records will be faxed to the receiving hatchery at least 3 days prior to shipment.

5. A minimum number of people shall work with these fish.

6. After any contact with these fish or water (e.g. picking up morts, brushing raceway), staff shall wash hands with soap and water or use a hand sanitizer.

General Sanitation

Background: The maintenance of a high standard of general sanitation is a proven method for minimizing disease outbreaks in both human and veterinary practices.

In our fish hatcheries, we have identified the following areas for general sanitation:

(1) Handling of fish mortalities from rearing units; (2) cleaning of rearing units between lots; and (3) cleaning of counters and floors in fish lab and production rooms.

Highest Risks

1. Bacterial pathogens in dead fish are at peak levels; therefore their handling can be considered a serious mode of disease transmission.

2. Parasites and bacterial pathogens may be transmitted in water and/or fish waste products.

3. Bacterial and viral pathogens may contaminate hands, floors and equipment in laboratory areas where sick fish are necropsied.

Fish Mortality Sanitation

1. Specific equipment (e.g., nets, buckets and vehicles) shall be designated for picking up dead fish. Buckets and nets used for this activity will be prominently labeled and not be used for any other activity.

2. A large bucket containing Virkon®Aquatic at 1-2% will be available for soaking nets between uses. Ideally, while a net is being used another one can be soaking for at least 5 -10 minutes before it is used again.

3. Mortalities will be removed twice daily from rearing units.

4. Mortalities shall be disposed of at the fish cleaning station or to a fenced "mort pit" at the George O. White Nursery for composting or burial. The remains from the fish cleaning station will be taken off-site to the state tree nursery for land application (as appropriate by permit).

5. Personnel shall wash their hands with soap and water or use hand sanitizer after collecting and disposing dead fish. Hand sanitizers shall be conveniently placed in vehicles.

6. The vehicle used to transport the dead fish shall be hosed out behind the hatchery building at the equipment cleaning area at least once weekly and disinfected (Figure 6). More frequent cleaning will be warranted if mortalities are high or there is spillage from buckets on to the bed of the vehicle.

Cleaning Rearing Units

1. Between lots of fish, rearing units shall be dewatered completely when possible.

2. On outdoor raceways, a power washer shall be used to remove organics from the sides and bottoms of the raceways. Units will then be left to dry for as long as possible.

3. Indoor tanks will be washed with a brush, sprayed with Virkon® Aquatic 0.5% and then allowed to dry before next use. If no chemicals are permitted, steam spraying for 5 minutes is recommended. Salt may be used as an abrasive. All units will be rinsed with clean water before use.

Fish Lab

1. When sick fish are brought into the lab (Hatchery Building; Figure 1) for necropsy, the following measures shall be taken to reduce contamination since both infected water and fluids often find their way to floors and counters .

    a. At the completion of work in the lab, the counters and floor will be sprayed or mopped with 500 ppm chlorine bleach (sodium hypochlorite) or 0.5% Virkon® Aquatic spray. Table 3 illustrates how to mix a proper bleach solution for disinfection (Note: the % active Na hypochlorite in household bleach varies by brand).

2. For routine laboratory cleaning and disinfecting of floors, 0.5% Virkon® Aquatic or 500 ppm chlorine bleach is recommended. Lower level, less corrosive disinfectants such as Roccal or Hyamin (quaternary ammonium compounds) or Lysol (phenol compound) are also satisfactory.

Table 3. How to calculate amount of household bleach (sodium hypochlorite) solution for a desired strength.		
$\frac{\text{(ppm Na hypochlorite desired) (gal of water) (128)}}{\text{\% of active Na hypochlorite (10,000)}}$	=	Ounces of household bleach needed
e.g. $\frac{\text{(500 ppm) (1 gal)(128)}}{\text{5.25\% (10,000)}}$	=	1.2 oz of 5.25% bleach per gallon of water

Basement Production Area for Fry (System #4, Figures 1 and 5)

1. At all times, areas to hatch eggs and rear fry shall be kept clean.

2. A foot bath using Virkon®Aquatic 1% shall be maintained at exits.

3. In-coming water to the basement production area will be treated with UV light. However, as noted in the introduction of this plan, there are problems with this system that need to be addressed. In particular, during periods of high water demand (all units being used), the UV unit severely restricts water flow. Consequently, it has to be turned off in order to meet the water requirements of the eggs, fry and fingerlings. Replacement of the current UV unit with a larger unit, or supplementing the current unit with an additional unit would not restrict water flow and would rectify this problem.

Weed Cutting Boat

Background: The weed cutting boat is used in streams to control aquatic vegetation at four MDC trout parks (Montauk, Maramec Spring, Roaring River and Bennett Spring). At Montauk, water from the stream where this boat is used also supplies water to some rearing units. Because this is a shared piece of equipment, we are concerned about the possible transfer of aquatic nuisance species, especially parasitic copepods, and unknown pathogens to Montauk Hatchery from other bodies of water where it is used.

Highest Risks

1. Zebra mussels
2. Nuisance aquatic plants
3. Parasitic copepods
4. Unknown pathogens carried in water

General Guidelines

1. Start with clean, dry boat.

2. After boat use, follow general cleaning protocol established for "Zebra Mussel Prevention" (Appendix 3) with the following recommendation: from among the possible disinfection methods indicated for controlling zebra mussels, power washing with hot water (>140° F for >10 sec or >176 F for >5 sec) or chlorine disinfection at 200 ppm then air drying (3-5 days) between uses at trout parks is recommended.

3. All personal equipment (waders, rain gear, rubber boots, gloves, etc.) exposed to stream water shall be sprayed with Virkon® Aquatic using a 0.5% solution before they are used again in hatchery waters.

Trout Vaccination

Background: Vaccination is a proven method to prevent and lessen the severity of many diseases. Both dip and injectable forms are available. At Montauk *Yersinia ruckeri* (enteric redmouth) vaccine is used on fingerlings (dip) and on broodstock (injection). *Aeromonas salmonicida* (furunculosis) vaccines are available and have been used on brown trout prior to their arrival at Montauk.

Highest Risks:

1. Ineffective vaccination, immunity not stimulated or excessive mortality of vaccinated fish.
2. Transfer of undesirable bacteria or virus particles to other fish via contaminated needle.
    a. *Aeromonas salmonicida*
    b. *Yersinia ruckeri*
    c. Other pathogens (bacteria, viruses-unknown at time of injection).
3. Risks to humans due to accidental self-injection of vaccine.

General Guidelines

1. Prior to vaccination, any abnormal fish shall be culled. These fish will not be vaccinated. In addition, staff shall be cautious when vaccinating any fish that have had a previous clinical outbreak of the disease for which they will be vaccinated. In this situation, staff shall consult the product label and wait the recommended length of time before vaccinating fish (For furunculosis this is 400 degree days = #days x mean water temperature in °C [e.g., @10° C or 50° F need 40 days]). The lot number of the vaccine, its expiration date, a copy of the vaccine label, date of vaccination, fish lot identification number and total number of fish vaccinated will be documented with each vaccination performed.

2. Injectable vaccines

<u>Human safety</u>

a. Set up a comfortable work station.

b. Safety guards shall be used to minimize the likelihood of accidental self injection.

c. Staff shall suspend the vaccination process once staff fatigue is apparent to minimize the likelihood of an accident. The process shall resume once staff are rested.

d. In the event of accidental human injection, staff shall seek emergency medical care immediately.

Fish

a. Fish shall be properly anesthetized with MS-222 to facilitate handling.

b. The injection needle shall be disinfected (rotated) frequently using one of the disinfectants listed in Table 4, discarded or autoclaved between lots of fish.

c. All equipment, buckets, table, gloves, etc. shall be disinfected at the end of the day.

3. Dip vaccines

a. Only clean sanitized dip nets shall be used to harvest fish from tanks and submerge fish in treatment containers. A new, disinfected bucket and net shall be used when the vaccine solution is changed.

b. All equipment, buckets, table, gloves, etc. shall be disinfected at the end of the day.

Table 4 . Disinfectants for vaccination needles only.

Disinfectant	Strength	Contact time	Rinse with water	Disadvantages
Virkon®Aquatic	2% (1:50 dilution)	5 min.	Yes	Corrosive to metal
Iodine	200 ppm	10 min.	Yes	Corrosive, less effective in hard water, stains
Chlorhexidine 2%	1-3 ounces	10 min.	Yes	Poor sporacidal activity
Alcohol	70%	30 min.	No-will evaporate	Flammable
Autoclave (will sterilize)	121° C and 15 psi	20 min at temperature		

Broodstock Management

Background: Shepherd of the Hills, Montauk, Roaring River and Bennett Spring hatcheries each maintain 2,000-4,000 rainbow trout as broodstock for egg production. Shepherd of the Hills Hatchery further utilizes "wild" brown trout from Lake Taneycomo as brood fish. At Montauk, brood fish are spawned outside adjacent to raceway #19 in System 3 (Figures 1 and 4) from September to the end of December. Approximately 1,000,000 eggs are collected annually.

Highest Risks

1. Viral pathogens (VHS, IHN, IPN)

2. Bacterial pathogens (bacterial kidney disease, coldwater disease, furunculosis)

General Guidelines

1. Annually, all broodstock shall be tested for VHS, IPN, IHN, whirling disease (*Myxobolus cerebralis*), bacterial kidney disease (*Renibacterium salmoninarum*), furunculosis and enteric redmouth per American Fisheries Society (AFS) blue book protocol.

2. At the time of spawning, staff will select only apparently healthy fish for spawning. In particular, fish with poor body condition, ulcerated skin, hemorrhagic skin, darkened skin, exophthalmia, a hemorrhagic vent or very pale gills shall be culled from the breeding population.

3. All eggs will be disinfected with 50-100 ppm iodine (Argentyne or Ovadine®) for at least 15- 30 minutes during water hardening to decrease the likelihood of a pathogen transfer from infected and non-clinical carrier fish. Water pH shall be maintained between 7.0 and 7.5 for optimal effectiveness. A detailed HACCP which describes in detail the egg disinfection procedure is provided in Appendix 3.

4. If furunculosis is a concern, it is recommended that the eggs be re-disinfected with 100 ppm iodine for 10 minutes during the eyed stage as an additional safeguard. However, this should not be done within 5 days of anticipated hatching.

Public Use

Background: Each year, 450,000 people visit Montauk State Park, of which 95,000 purchase tags to fish. They come from all over the world. The public is welcome to freely explore areas around our outdoor raceways and guided tours are provided inside of hatching areas. MDC encourages educational interaction with the public at all fish hatcheries.

In addition to humans, our fish hatcheries also have wildlife visitors. Wildlife frequenting facilities include great blue herons, bald eagles, skunks, raccoons, muskrats, possums and various waterfowl. Wildlife not only preys upon fish but can also transfer diseased fish from one area to another and regurgitate partially eaten food. They are recognized as biological vectors of disease.

Highest Risks

Humans

1. Although the risk is ranked as low, footwear and hands of visitors may transfer undesirable pathogens, parasites, or aquatic nuisance species (e.g. whirling disease spores, zebra mussels) onto facilities (muddy shoes) or between rearing units (if hands are put in the water).

2. Trash or rocks thrown in water may be consumed by fish who mistake it for food.

Wildlife

1. Mechanical transfer of diseased fish or aquatic nuisance species.

General Guidelines-Humans

1. Public traffic around outdoor rearing areas is unavoidable.

2. Where possible, fish at greatest risk of disease (youngest fish or most sensitive species) will be located as far away from frequent foot traffic as possible. System 1 (Figure 5) most closely meets this criterion. Public access is restricted in this area.

3. Visitors inside of hatching areas (System #4) shall be accompanied at all times and shall step into a foot bath at the bottom of the stairs upon entering and leaving this area (Figure 5). Special displays will be used at certain vantage points to decrease the amount of foot traffic and to minimize the disturbance to these fish.

4. If a rearing unit is experiencing high mortality, all activity will be minimized in this area.

General Guidelines-Wildlife

1. The following steps may be undertaken to discourage nuisance wildlife residence at MDC hatcheries.

a. Raceways and mortality disposal areas will be fenced, sides and top.

b. Dead fish will be removed from rearing units twice daily.

c. The practice of feeding dead fish to wildlife will be discouraged.

d. Feed storage areas will be kept clean and tidy.

General Disease and Aquatic Nuisance Species Surveillance

Background: Implementation of a surveillance program will provide a means of detecting the presence of aquatic nuisance species and pathogens for early intervention and help provide bench marks for eradication measures.

Highest Risks

1. Viral pathogens: VHS, IPN, IHN

2. Parasites: whirling disease, parasitic copepods (*Salmincola californiensis)*, ich, trematodes, *Trichodina*, *Costia*, *Chilodonella*, and *Epistylis*

3. Bacteria: *Aeromonas salmonicida, Yersinia ruckeri, Flavobacterium psychrophilum, Flavobacterium columnare, Aeromonas spp., Pseudomonas spp., Renibacterium salmoninarum, Flavobacterium branchiophilum*

4. Aquatic nuisance species: zebra mussel, New Zealand mud snails, Quagga mussels, etc.

General Guidelines

1. Good staff education is the number one preventive measure for disease surveillance. Education will provide a means for staff to recognize problems and take steps to correct them.

a. Recommend that each staff member take the Basic Fish Health class provided by MDC.

b. If there is interest, the MDC Aquatic Animal Health Specialist will be available to provide additional on-site training or refreshing of fish diagnostic techniques.

2. Artificial substrates consisting of layers of discarded plastic signs for detecting zebra mussels shall be placed in Montauk Lake at the intake structure for Lake Raceways (System #3), and in the Current River at the intake structure for the Mill Raceways (System #2) (Figure 6). Veliger monitoring will also be conducted at these locations. Figure 7 illustrates a sampler covered with zebra mussels. Directions for construction of a monitoring device are provided in Appendix 4. The sampler will be visually monitored at least once every 3 months.

3. The MDC Aquatic Animal Health Specialist will conduct annual testing for viruses (IPN, IHN, VHS), whirling disease, parasitic copepods, BKD, enteric redmouth, and furunculosis at all MDC fish hatcheries.

4. Fish displaying abnormal behaviors (going off feed, changes to skin color, reddened fins, increased mortalities, etc.) will be promptly evaluated (at a minimum, by general external appearance, skin scrape and gill biopsy) on-site and treated appropriately. If initial therapy is unsuccessful or if additional tests are needed which are not available on-site (e.g. bacterial culture, histopathology) they will be referred to the MDC Aquatic Animal Health Specialist for further evaluation.

5. Mortality records in writing will be maintained on a daily basis for each rearing unit. Periodically, these records will be sent to other fish hatcheries during fish transfers and will provide a means of monitoring fish health.

Figure 7. Artificial sampler covered with zebra mussels 7 months after placement in a zebra mussel positive body of water (from Brian McKeage, MDC).

Budgeting Considerations and Summary

To fully implement this plan, there will be a need for additional funding over our current budget. These funds will be needed for additional operational expenses, to purchase additional equipment and for educational materials such as brochures, signs and displays.

Additional operational funds needed are:

1. Additional $2,000 annually for salt.
2. Additional $3,000 annually for Virkon® Aquatic and other chemical therapeutics and disinfectants.
3. Additional $1,500 initially for waders, gloves and rain gear to be used by staff from other facilities.
4. Additional $3,500 initially for nets, brushes, brooms, foot baths and other cleaning supplies designated for each system.
5. $12,000 for a small construction request in FY2011 to replace our current UV sterilizer or install an additional UV sterilizer for the basement area (System #4, Figure 5).
6. $1,500 initially for development of educational material on the importance of biosecurity.

Again, there are two main objectives of the Montauk Biosecurity Plan. The first will be to reduce the chance of importing or exporting pathogens to or from other hatcheries and waters. The second is to improve the ability to reduce disease outbreaks, isolate pathogens and reduce the risk of spreading them throughout the system.

Appendix 1. Fish Transfer Information Sheet

(Submit via email at least 3 days prior to shipment)

Today's date: _____

Anticipated shipment date: _____

From: ☐Bennett ☐Maramec ☐Montauk ☐Roaring River ☐Shepherd ☐Other: _____

To: ☐Bennett ☐Maramec ☐Montauk ☐Roaring River ☐Shepherd ☐Other: _____

Lot Designation: _____ From Raceway/Unit: _____

Lot History

Mortality record for last 15 days provided (see page 2)? ☐ Yes ☐ No
Fish taken off feed 3 days prior to shipment? ☐ Yes ☐ No

The following aquatic nuisance species occur in this hatchery's watershed:
☐ Zebra mussels ☐ Parasitic copepods ☐ Rusty crayfish ☐ Quagga mussels
☐ New Zealand mud snails ☐ Other: _____

General Health within last 30 days: ☐ Excellent ☐ Good ☐ Fair ☐ Poor
(Basis of this classification: ☐ Mortality records ☐ Observation/feed intake ☐ Necropsy: gills, skin, organs)

If fair or poor: what problems were observed or suspected? _____
Any chronic problems? _____

Therapeutic Used in last 30 days	Yes √	Date Treated	Why treated?	Results or Comments (cured problem; partial, little or no improvement)
None	☐			
Aquaflor	☐			
Chloramine-T	☐			
Copper sulfate	☐			
Formalin	☐			
Immersion OTC	☐			
MS-222	☐			
Oxytetracycline	☐			
Perox-Aid	☐			
Romet	☐			
Salt	☐			
Vinegar	☐			

Mortality Record Previous 15 days

Date		
Month	Day	Daily Mortalities

Appendix 2. .Zebra Mussel Prevention Policy

The Missouri Department of Conservation will work to prevent the spread of zebra mussels from infested waters to uninfested waters.

est. 10/05

PROCEDURES

● RESOURCE THREAT

Zebra mussels can clog power plants, industrial and public drinking water intakes, foul boat hulls, decimate populations of freshwater mussels and other native aquatic organisms, impact fisheries and disrupt aquatic ecosystem functions. Economic impacts of zebra mussels in North America are estimated to be in the billions of dollars. Because of the ease with which microscopic larval zebra mussels may be transported by the public, it may take several years to detect an infestation. Avoiding known infested areas, or staging equipment use such that waters known, or suspected to be infested, are visited last, will help prevent the spread of zebra mussels. However, boats, equipment, and gear must be decontaminated prior to use in different waters. Personnel will take reasonable precautions to avoid exposure of equipment, facilities, and other waters to zebra mussels.

● PUBLIC OUTREACH AND EDUCATION

Increased public outreach and education will enhance understanding of the potential problems associated with zebra mussels and the measures that may help deter their expansion. Signs should be posted at all MDC owned and managed boat ramps highlighting the potential problems associated with zebra mussels. Information should be distributed through our state, federal and non-governmental agency partners, MDC managed waterfowl areas, trapping associates, sport fishing groups, marinas, lake associations, Department offices and Nature Centers, media outlets and to other water users in Missouri.

● EQUIPMENT DECONTAMINATION PROCEDURES

Appropriate safeguards to prevent the transfer of zebra mussels from one waterbody to another are mandatory and include inspection, treatment, and, if possible, avoidance. The following steps detail equipment decontamination procedures:

1. Thoroughly inspect boats (hulls, drive units, trim plates, transducers), trailers and components (rollers, bunk boards, axles, etc.), equipment (i.e., water pumps, hatchery equipment, siphons, nets, ropes, traps, etc.), and machinery (tractors, bulldozers, etc.) for adult zebra mussels. Pay close attention to nooks, crannies and other inconspicuous places (i.e., around the motor housing, trim tabs, and water intake screens, or pump fittings). All trash, mud, vegetation, and suspected zebra mussels should be removed and properly disposed of in the trash. Immediately report suspected occurrences of zebra mussels to the Invasive Species Coordinator.

2. Carpeted bunks and runners on existing boat trailers should be replaced with poly, plastic or wooden bunks as soon as practical; boat trailers regularly moved between known zebra mussel infested waters and other waters should have carpeted bunks and runners replaced immediately. As available, future boat trailers should be purchased with poly/plastic/wooden bunks.

3. All water should be drained from boats, trailers, motors, live wells, bilges, transom wells, holding tanks and live wells, water pumps, pipes, and other equipment prior to leaving a waterway. Pay particular attention to boat hulls under installed decking. Drain as much water as possible from equipment such as lower motor units and portable pumps.

4. Any boat, trailer, tank, equipment, machinery, gear, or net transferred from one body of water into a different body of water or from known infested waters to potentially infested waters must be decontaminated using one of the treatments in Table 1 prior to being used in a new body of water. Equipment decontamination procedures should be completed when moving equipment from infested areas of a water body to uninfested areas of the same water body. If boats, nets, and other equipment are only used in one body of water, cleaning between uses is not necessary, but these boats, nets, and other equipment MUST be clearly labeled for use in that body of water ONLY. Periodic cleaning and decontamination (i.e., during winterization or other maintenance) should be conducted to prevent costly repairs. If management or research activities require this equipment to be moved in the future, decontamination procedures will be implemented.

● HATCHERY PRECAUTIONS

Best management practices should be used to protect equipment and facilities and to reduce the opportunity for the spread of zebra mussels to uninfested areas. Introductions of zebra mussels into MDC fish hatcheries or water supply sources would have devastating impacts upon hatchery infrastructure. If infested, hatcheries would then be a possible mechanism for transporting the organisms to uninfested waters. Therefore, the following precautionary measures will be enacted by MDC fish hatcheries:

1. All attempts will be made to secure fish from sources known to be free of zebra mussels (veligers and adults) (see map at http://intranet/Documents/17407.pdf)

2. All fish and eggs exposed to surface water coming into or leaving any of MDC's hatcheries or other facilities and any fish procured through contract or other means from outside sources must be treated during transportation using one of the treatments in Table 3. The only exception will be for fish that are stocked into the same water supply that is used by the hatchery (e.g., trout stocked in Bennett Spring branch by Bennett Spring Hatchery staff) and for selected species of conservation concern.

3. Specific limitations may be applied to native mussel and hellbender culture, and other species of conservation concern, on a case-by-case basis.

4. Some species or life stages of fish or other aquatic organisms may be less tolerant of chemical treatments. For these species or life stages whose chemical tolerances are unknown, bioassays must be performed prior to large scale use of the prescribed treatments listed below. Until these bioassays are conducted, brood stock of these species will only be obtained from waters known to be free of zebra mussels.

Table 1. Zebra Mussel Disinfectants and Usage Guidelines for Boats and Equipment

Disinfectant	**Concentration**	**Contact Time**	**Usage Guidelines, Safety Precautions, Drawbacks**
Vinegar	100%	20 min	Use appropriate personal protective equipment (PPE) and caution. Stay upwind of the spray. Is corrosive to metal and toxic to fish at this concentration, so thoroughly rinse with tap water or water from the next lake or river after disinfection. Ensure that solution does not run-off directly into waterways.
Chlorine	200 ppm	10 min	Use appropriate PPE and caution. Stay upwind of the spray. Is corrosive to metal and rubber and toxic to fish at this concentration, so neutralize with 800 ppm sodium thiosulfate and rinse thoroughly with tap water or water from the next lake or river. Ensure that solution does not run-off directly into waterways.
Power wash with hot water	>104° F	20 min	Use appropriate PPE and caution when using hot water due to possibility of burns/scalding. Temperature and contact times are crucial, as efficiency is weather dependent. Most effective when used in conjunction with air drying (see below). Power wash with hot water, including thoroughly flushing lower motor unit.
Freezing	<32° F	24 hrs	Boats, gear, and equipment should be thoroughly frozen. Ambient air temperature should remain below freezing for the entire contact time. No safety precautions.
Air drying	N/A	3-5 days in hot sun 48 hrs in hot sun	Must dry completely to be effective. Most effective when used in conjunction with hot water (see above). To be used for small nets, gear, pumps, etc., *ONLY AFTER* power washing with hot (104°) water for appropriate contact time.
Salt Bath	1%	24 hrs	Due to the long contact time, may only be used as a bath solution and not sprayed. To be used only for pieces of equipment, gear, and nets that can be completely immersed in the solution.

Table 2. Disinfectant Amounts to Make Needed Concentrations					
Disinfectant	**1 gallon**	**2 gallons**	**5 gallons**	**20 gallons**	**100 gallons**
100% Vinegar	1 gal	2 gal	5 gal	20 gal	100 gal
200 ppm Chlorine (household bleach, 5.25% Chlorine)	0.5 ounce (15 ml)	1.0 ounce (30 ml)	2.5 ounces (75 ml)	11.0 ounces (300 ml)	6 1/3 cups (1.5 L)
200 ppm Chlorine (HTH granular)	0.04 ounce (1.2 g)	0.08 ounce (2.4 g)	0.2 ounce (6 g)	0.8 ounce (24 g)	4.2 ounces (120 g)
800 ppm Sodium Thiosulfate	0.1 ounce (3 g)	0.2 ounce (6 g)	0.5 ounce (15 g)	2.1 ounces (60 g)	10.6 ounces (300 g)
1% Salt Bath (as NaCl)	1/8 cup	1/4 cup	2/3 cup	2 2/3 cups	13 1/3 cups

Notes:

1. Air drying and hot water are most effective when used in conjunction with each other because their effectiveness is highly dependent upon ambient temperatures and contact times. As needed, hot water wash units should be made available at selected Department facilities.
2. Household bleach (5.25% chlorine) and vinegar can be purchased from grocery or convenience stores. HTH granular chlorine (70% calcium hypochlorite) and Sodium Thiosulfate can be purchased at pool supply stores or chemical companies.
3. All bilges and hidden areas under boat decks must be thoroughly treated as described above.
4. Source: WI DNR (2007) *Equipment Disinfection Protocol for Invasive Species and Viruses*.

Table 3. Hatchery/Fish/Aquatic Organism Zebra Mussel Treatments and Usage Guidelines			
Treatment	**Concentration**	**Contact Time**	**Usage Guidelines/Comments**
NaCl	20,000 ppm	2 hrs	Used for striped bass only. Treatment conducted during transport.
KCl/formalin	750 ppm KCl 25 ppm formalin	1 hr 2 hrs	Used for all other fish species and eggs. Fish and hauling water are pretreated for 1 hour with 750 ppm KCl, followed by a 2 hour treatment with 25 ppm formalin during transport. *DO NOT* treat fish with NaCl to counteract shock, as this decreases the effectiveness of the treatment.

Notes:

1. All fish, including those used in aquaria at nature centers, fairs, etc., are to be treated for zebra mussels while in transit.

2. Treatment concentrations and contact times that are currently exceeded during normal aquaculture operations (e.g., egg hardening and shipping) should be considered effective.
3. Some species or life stages of fish or other aquatic organisms may be less tolerant of chemical treatments. For these species or life stages whose chemical tolerances are unknown, bioassays must be performed prior to large scale use of the treatments listed above.
4. For species with known intolerances to recommended zebra mussel treatments, modifications of hatchery assignments, increased use of well water, UV treatment, sand filtration, and other system modifications or treatment/avoidance measures may be needed and should be considered on a case-by-case basis with the involvement and approval of Division Chiefs and the Invasive Species Coordinator.
5. Sources: IA DNR *Fairport Fish Hatchery ANS-HACCP*, Edwards *et al*. 2000.

Appendix 3. Egg disinfection HACCP for Salmonid eggs.

HACCP Step 1 - Activity Description	
Facility: Coldwater Hatcheries	**Site**: Missouri Department of Conservation: Cold-water Fish Hatcheries
Project Coordinator:	**Project Description**: Rainbow and Brown Trout Egg Collection and Disinfection
Site Manager:	
Address:	
Phone:	

Project Description (Who, What, Where, When, How & Why)
During fall and spring, rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo grutta) are spawned at four MDC fish hatcheries (Shepherd of the Hills, Bennett Spring, Montauk and Roaring River). Each hatchery maintains its own population of rainbow trout for breeding purposes year around. Eggs are either air spawned or hand-stripped from females. The eggs are collected in pans then fertilized with milt from male fish. After water hardening the fertilized eggs are placed in an incubator where they develop. At the eyed stage some fish may be shipped to other facilities for hatching. Others will remain on site where they will be hatched and grown-out. At 51° F hatching will take approximately 31 days. Pathogens of greatest concern for transmission during egg collections include those viruses (VHS, IPN, and IHN) and bacterias (*Aeromonas salmonicida* and *Flavobacterium psychrophilum*, *Renibacterium salmoninarum*) that can contaminate the surface of the egg or be carried within the egg.

HACCP Step 2 - Potential Hazard Identification

Vertebrates:
Invertebrates:
Plants:
Other Biologics: Viral and Bacterial Pathogens
Others:

HACCP Step 3 - Flow Diagram

Task # 1	Broodstock are gathered into holding pens.

Task # 2	Fish are anesthetized with MS-222

Task # 3	Ripe fish are selected for spawning

Task # 4	Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are transferred to a dry stainless steel bowl.

Task # 5	Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.

Task # 6	Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.

Task # 7	Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.

Task # 8	The disinfection solution is drained and the eggs transferred to incubators.

Task # 9	Eggs are monitored daily for development, mortality and fungus. Dead

	eggs are removed

Task # 10	Eyed eggs may be transferred to another hatchery or allowed to hatch on site where they are reared in troughs for the next several months.

HACCP Step 4 - Hazard Analysis					
Task	**Hazard**	**Probable?**	**Justification**	**Control Measures**	**CCP?**
Broodstock are gathered into holding pens.	Other Biologic: Viral and Bacterial Pathogens	Yes	Broodstock may be symptomatic or asymptomatic carriers of pathogens. Water source for hatchery is "unprotected" or contains fish so infection is possible despite tests on population.	Only a population of broodstock previously tested and found negative for VHS, IPN, and IHN will be used. All equipment used to handle fish shall be disinfected after use.	Yes
Fish are anesthetized with MS-222	Other Biologic: Viral and Bacterial Pathogens	No	No pathogens are introduced in this step.		No
Ripe fish are selected for spawning	Other Biologic: Viral and Bacterial Pathogens	Yes	Some fish may be symptomatic or asymptomatic carriers of pathogens	Any apparently unhealthy fish will be culled from the breeding population.	Yes
Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are	Other Biologic: Viral and Bacterial Pathogens	Yes	Pathogens may be carried in skin mucous, sex products or inside of the egg. Other contamination could occur though use of a contaminated needle or dirty bowls.	Ovarian fluid will be collected and tested for viruses. Needles used for air spawning be changed frequently and disinfected between uses. Bowls will be cleaned before	Yes

transferred to a dry stainless steel bowl.				use and changed between egg take groups.	
Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.	Other Biologic: Viral and Bacterial Pathogens	Yes	Pathogens may be carried in skin mucous, milt or on surface of dirty bowls.	Symptomatic fish are culled in a previous step. Clean bowls are used in task #4.	Yes
Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.	Other Biologic: Viral and Bacterial Pathogens	Yes	A dirty container or contaminated water may harbor pathogens. Pores of egg are open and susceptible to entry of pathogens.	Use oxygenated distilled or u/v sterilized water and make sure all containers are disinfected before use.	Yes
Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket, the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If	Other Biologic: Viral and Bacterial Pathogens	Yes	Improper concentration of iodine, improper pH, insufficient contact time or poor contact with the egg surface may prevent proper disinfection or cause excessive mortality of eggs	Measure iodine carefully (50-100 ppm) and spread eggs out horizontally to improve surface contact with iodine. Use a buffered approved iodine chemical (Argentyne, Ovadine®). Carefully time disinfection	Yes

eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.				process. Ideal pH is 7-7.5. All equipment used to count eggs is disinfected before use and between lots.	
The disinfection solution is drained and the eggs are transferred to incubators	Other Biologic: Viral and Bacterial Pathogens	Yes	Disease transmission is possible if incubator is contaminated.	Disinfect incubator before use.	Yes
Eggs are monitored daily for development, mortality and fungus. Dead eggs are removed	Other Biologic: Viral, Fungal and Bacterial Pathogens	Yes	Development and survival of eggs is dependent on environmental conditions. Decay of dead eggs uses up oxygen and promotes growth of fungus.	If fungus develops, treat eggs with formalin or perox-aid. Remove dead eggs to maintain sanitary environment for developing eggs	Yes
Eyed eggs may be transferred to another hatchery or allowed to hatch on site where they are reared in troughs for the next several months.	Other Biologic: Viral and Bacterial Pathogens	Yes	If eggs were improperly disinfected in step 7, eggs could still be contaminated.	If eggs are shipped, receiving hatchery should soak eggs in water for 30-60 min to rehydrate them, then re-disinfect eggs for 10 min at 100 ppm iodine. This second disinfection provides	Yes

				additional security.	

HACCP Step 5 - HACCP Plan

Critical Control Point #1:
Task # 1: Broodstock are gathered into holding pens.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Only a population of broodstock previously tested and found negative for VHS, IPN, and IHN will be used. All equipment used to handle fish shall be disinfected after use.

Limits for Control Measures:
If hatchery water supply is not "secure" fish could contract pathogen at any time after screening tests are performed; broodstock may be symptomatic or asymptomatic carriers of viruses or bacteria and may shed pathogens intermittently or have too low of a level to detect.

Monitoring: What?
Viruses (IPN, IHN, VHS), bacteria (*Yersinia ruckeri, Aeromonas salmonicida, Renibacterium salmoninarum*), and parasites (*Salmincola spp, Myxobolus cerebralis)*

Monitoring: How?
Samples of tissues are collected for viral analysis; bacteria is cultured from the posterior kidney; and cartilage is tested for Whirling Disease. Parasitic copepods are detected by visual observation.

Monitoring: Frequency?
Tests are performed annually.

Monitoring: Who?
MDC Fish Health Specialist will collect screening tests on population.

Evaluation & Corrective Actions:
Depopulate or quarantine hatchery if broodstock are found to harbor a serious pathogenic virus.

Supporting Documentation: AFS Blue book; Manual of Diagnostic Tests for Aquatic Animals OIE).

Critical Control Point #2:
Task # 3: Ripe fish are selected for spawning

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Any apparently unhealthy fish will be culled from the breeding population.

Limits for Control Measures:
Some fish are asymptomatic carriers and do not display any clinical signs of infection yet can shed the pathogen when stressed.

Monitoring: What?
Monitoring of daily health shall include detection of abnormal behaviors, mortality, physical defects or anorexia.

Monitoring: How?
Visual observation

Monitoring: Frequency?
Daily basis.

Monitoring: Who?
Hatchery staff will monitor daily health.

Evaluation & Corrective Actions:
Individual fish can be tested for virus, bacteria or parasites by Aquatic Animal Health Specialist.

Supporting Documentation:

Critical Control Point #3:

Task # 4: Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are transferred to a dry stainless steel bowl.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Ovarian fluid will be collected and tested for viruses. Needles used for air spawning be changed frequently and disinfected between uses. Bowls will be clean before use and changed between egg take groups.

Limits for Control Measures:
Ovarian fluid from every fish cannot be checked for viruses only a representative sample from population. A proper disinfectant and contact time are needed to adequately kill virus and bacteria on needles.

Monitoring: What?
Ovarian fluid is tested for VHS, IPN, and IHN.

Monitoring: How?
Ovarian fluid is collected from 60 female fish during spawning.

Monitoring: Frequency?
Annually.

Monitoring: Who?
MDC Aquatic Animal Health Specialist: ovarian fluid; Hatchery staff: spawning process.

Evaluation & Corrective Actions:
None

Supporting Documentation:

Critical Control Point #5:

Task # 5: Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Symptomatic fish are culled in a previous step. Clean bowls are used in task #4.

Limits for Control Measures:
A proper disinfectant and contact time is needed to clean a bowl.

Monitoring: What?
Cleanliness of bowl.

Monitoring: How?
Visual observation

Monitoring: Frequency?
All bowls should be clean before spawning operations begin in previous step.

Monitoring: Who?
Hatchery staff (previous step)

Evaluation & Corrective Actions:
None

Supporting Documentation: John Shrable, Daniel Abeyta, Jim McFall, David Noble. Developments in Fish Culture: Compare fertilization success when mixing eggs and milt by hand stirring versus pouring from one pan to another, USFWS, Ennis National Fish Hatchery, September 9, 1998 (unpublished).

Critical Control Point #5:

Task # 6: Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Use chilled oxygenated distilled or u/v sterilized water and make sure all containers are disinfected before use.

Limits for Control Measures:
It is difficult to detect and monitor pathogens present in "untreated" water.

Monitoring: What?
Quality of water used to harden eggs.

Monitoring: How?
Visual observation and history.

Monitoring: Frequency?
Check before use.

Monitoring: Who?
Hatchery staff

Evaluation & Corrective Actions:
Locate a different water source if necessary.

Supporting Documentation:

Critical Control Point #6:

Task # 7: Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket, the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Measure iodine carefully (50-100 ppm) and spread eggs out horizontally to improve surface contact with iodine. Use a buffered approved iodine chemical (Argentyne, Ovadine®) and distilled oxygenated water. Carefully time disinfection process. Ideal pH is 7-7.5. All egg counting devices shall be disinfected with iodine prior to use and between lots of eggs.

Limits for Control Measures:
Water quality will affect activity of iodine; pH will impact egg sensitivity to iodine (survival of egg); success depends on pathogen susceptibility to iodine; and quantity and distribution of eggs will affect degree of IO surface contact.

Monitoring: What?
The concentration of iodine is monitored, the length of time of the treatment, and pH of solution are monitored

Monitoring: How?
Monitor iodine levels visually-as color fades, add more iodine; use an iodine test strip; use a stop watch for timing; use a pH meter or chemical test kit for PH. Egg continuing devices shall be visually clean before use.

Monitoring: Frequency?
Monitor constantly during disinfection. Monitor equipment before use.

Monitoring: Who?
Hatchery staff.

Evaluation & Corrective Actions:
If disinfection is not satisfactory, eggs can be re-disinfected for 15-30 minutes at 50-100 ppm after water hardening up to 5 days before hatching.

Supporting Documentation: "Iodophor disinfection of fish eggs" US Fish & Wildlife service aquatic handbook; product labels.

Critical Control Point #7:
Task # 8: The disinfection solution is drained and the eggs are transferred to incubators

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Disinfect incubator before use.

Limits for Control Measures:
Proper disinfection technique for incubators.

Monitoring: What?
Cleanliness of incubator.

Monitoring: How?
Visual observation

Monitoring: Frequency?
Check before use.

Monitoring: Who?
Hatchery staff

Evaluation & Corrective Actions:
If dirty incubator, select another unit that is clean.

Supporting Documentation:

Critical Control Point #8:
Task # 9: Eggs are monitored daily for development, mortality and fungus. Dead eggs are removed

Significant Hazards:
Other Biologic: Viral, Fungal and Bacterial Pathogens

Control Measures:

If fungus develops, treat them with formalin or Perox-aid. Remove dead eggs to maintain sanitary environment for developing eggs

Limits for Control Measures:
Amount of available labor will dictate how frequently dead eggs can be removed; water quality will also impact success of egg development.

Monitoring: What?
Staff will monitor the color of the egg for detecting viability. White or black eggs are dead. Fuzz indicates fungus.

Monitoring: How?
Visual observation.

Monitoring: Frequency?
Daily basis during egg development.

Monitoring: Who?
Hatchery staff.

Evaluation & Corrective Actions:
None other than destroying the unit of infected eggs.

Supporting Documentation:

Critical Control Point #9:

Task # 10: Eyed eggs may be transferred to another hatchery or allowed to hatch on-site where they will be reared in troughs for the next several months.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
If eggs are shipped, receiving hatchery should soak eggs in water for 30-60 min to rehydrate the eggs, then re-disinfect eggs for 10 min at 100 ppm iodine. This second disinfection provides additional security.

Limits for Control Measures:
If initial disinfection was not successful or eggs were contaminated in hatchery water, eggs may still harbor pathogens. Iodine disinfection should not be done within 5 days of hatching since this may kill the developing embryo or cause premature hatching

Monitoring: What?
Staff will monitor iodine treatment as in task #7, critical control point # 6.

Monitoring: How?
See critical control point #6

Monitoring: Frequency?
See critical control point #6.

Monitoring: Who?

Hatchery staff of receiving facility	
Evaluation & Corrective Actions: None	
Supporting Documentation:	
Facility: Coldwater Hatcheries	**Activity**: Rainbow Trout Egg Collection and Disinfection
Address:	
Signature:	**Date**:

HACCP Checklist:
Rainbow and Brown Trout Egg Collection and Disinfection

Facility

Site Missouri Department of Conservation: Cold water Fish Hatcheries

Coordinator

Manager

Address ,

☐ **Task # 1: Broodstock are gathered into holding pens. CRITICAL CONTROL POINT**

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Only a population of broodstock previously tested and found negative for VHS, IPN, IHN will be used. All equipment used to handle fish shall be disinfected after use.
- ☐ Control limits were maintained
  Control Limits: If hatchery water supply is not "secure" fish could contract pathogen at any time after screening tests are performed; broodstock may be symptomatic or asymptomatic carriers of viruses or bacteria and may shed pathogens intermittently or have too low of a level to detect..
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: Depopulate or quarantine hatchery if broodstock are found to harbor a serious pathogenic virus.

☐ **Task # 2: Fish are anesthetized with MS-222**

☐ **Task # 3: Ripe fish are selected for spawning**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Any apparently unhealthy fish will be culled from the breeding population.
- ☐ Control limits were maintained
  Control Limits: Some fish are asymptomatic carriers and do not display any clinical signs of infection yet can shed the pathogen when stressed.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: Individual fish can be tested for virus, bacteria or parasites by Aquatic Animal Health Specialist.

☐ **Task # 4: Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are transferred to a dry stainless steel bowl.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Ovarian fluid will be collected and tested for viruses. Needles used for air spawning be changed frequently and disinfected between uses. Bowls will be clean before use and changed between egg take groups.
- ☐ Control limits were maintained
  Control Limits: Ovarian fluid from every fish cannot be checked for viruses only a representative sample from population. A proper disinfectant and contact time are needed to adequately kill virus and bacteria on needles.

- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None

☐ **Task # 5: Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Symptomatic fish are culled in a previous step. Clean bowls are used in task #4.
- ☐ Control limits were maintained
  Control Limits: A proper disinfectant and contact time is needed to clean a bowl.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None

☐ **Task # 6: Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Use oxygenated distilled or u/v sterilized water and make sure all containers are disinfected before use.
- ☐ Control limits were maintained
  Control Limits: It is difficult to detect and monitor pathogens present in "untreated" water.
- ☐ Corrective actions were (performed if necessary)

Corrective Actions: Locate a different water source if necessary.

☐ **Task # 7: Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket, the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Measure iodine carefully (50-100 ppm) and spread eggs out horizontally to improve surface contact with iodine. Use buffered, approved iodine chemicals (Argentyne, Ovadine®). Carefully time disinfection process. Ideal pH is 7-7.5.
- ☐ Control limits were maintained
  Control Limits: Water quality will affect activity of iodine; pH will impact egg sensitivity to iodine (survival of egg); susceptibility of pathogen to iodine; and quantity and distribution of eggs will affect degree of IO surface contact.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: If disinfection is not satisfactory, eggs can be re-disinfected for 10-30 minutes at 50-100 ppm after water hardening up to 5 days before hatching.

☐ **Task # 8: The disinfection solution is drained and the eggs are transferred to incubators.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Disinfect incubator before use.
- ☐ Control limits were maintained
  Control Limits: Proper disinfection technique for incubators.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: If dirty incubator, select another unit that is clean.

☐ **Task # 9: Eggs are monitored daily for development, mortality and fungus. Dead eggs are removed**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral, Fungal and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: If fungus develops, treat them with formalin or Perox-aid. Remove dead eggs to maintain sanitary environment for developing eggs
- ☐ Control limits were maintained
  Control Limits: Amount of available labor will dictate how frequent dead eggs can be removed; water quality will also impact success of egg development.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None other than destroying the unit of infected eggs.

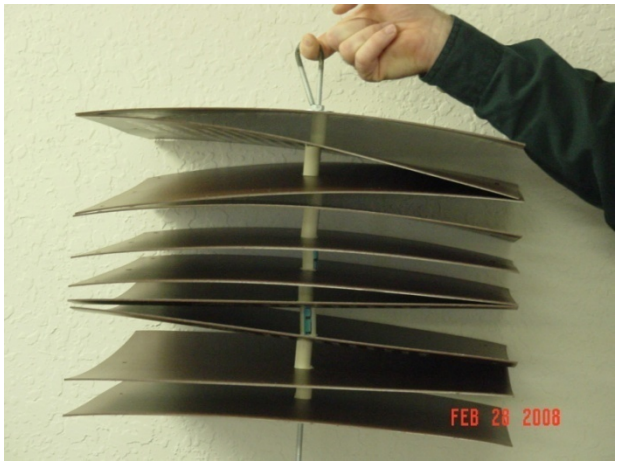
☐ **Task # 10: Eyed eggs may be transferred to another hatchery or allowed to hatch on site where they are reared in troughs for the next several months.**

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: If eggs are shipped, receiving hatchery should soak eggs in water for 30-60 min, and then re-disinfect eggs for 10 min at 100 ppm iodine. This second disinfection provides additional security.
- ☐ Control limits were maintained
  Control Limits: If initial disinfection was not successful or eggs were contaminated in hatchery water, eggs may still harbor pathogens. Iodine disinfection should not be done within 5 days of hatching since this may kill the developing embryo or cause premature hatching.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None

Appendix 4. Directions for construction of zebra mussel artificial substrate sampler. Source: Brian McKeage, MDC, Missouri River Unit, Columbia, email 3/23/09.

They are pretty simple to make. All you need is 10 old Department plastic signs, a piece of 5/16" all thread with a loop bent in the top, 1" pieces of ½"cpvc pipe and a nut and washer. Drill a hole through the signs, place the signs on the all thread with a loop, put the cpvc pipe pieces in to space out the signs and finish by putting the nut and washer on after the last sign. You can then tie a rope to the loop and hang the monitor up in the water body.

Scott has the picture of the monitor before we put it in the water.



Thanks,
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